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Synergism by Propynyl Aryl Ethers in Permethrin-Resistant Tobacco Budworm Larvae, *Heliothis virescens*

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Abstract: Synergists were used to diagnose possible mechanisms of permethrin resistance in permethrin-selected strains of the tobacco budworm, *Heliothis virescens* (F.). In addition to permethrin, these strains of the tobacco budworm were resistant to α-cyano-pyrethroid insecticides, organophosphorus insecticides and DDT. The monooxygenase-inhibiting prop-2-ynyl aryl ethers were the only effective synergists of permethrin among 16 candidates tested. The most effective synergist was 1,2,4-trichloro-3-(2-propynyloxy)benzene. Piperonyl butoxide, a common monooxygenase-inhibiting synergist in other species and tobacco budworm strains, was inactive. These results suggested the presence and contribution of an unusual monooxygenase in the enzymatic detoxication of permethrin. DDT cross-resistance, which was not synergized, and broad pyrethroid cross-resistance supported previous evidence for target site insensitivity as a second pyrethroid-resistance mechanism in these strains. The actions of *S,S,S*-tributyl phosphorotrithioate (TBPT) and triphenyl phosphate (TPP) suggested that hydrolytic detoxication, important in methyl parathion-resistance tobacco budworm strains, had little or no role in conferring pyrethroid resistance in these strains.

Key words: cotton pest, insecticide resistance, monooxygenase, permethrin, propargyl ether, pyrethroid, synergistic mixture.

1 INTRODUCTION

Enzymatic detoxication of pesticides is one of several common mechanisms by which pests develop pesticide resistance. To identify detoxication in a pest that might possess any of several mechanisms, diagnostic synergists may be applied in mixtures with the insecticide of interest.[1] A mixture containing an effective synergist will kill a resistant insect because the synergist inhibits enzymatic detoxication and allows the insecticide to reach its target. Propynyl aryl ethers, which were discovered as carbamate insecticide synergists,[2] also synergized the first synthetic pyrethroid, allethrin, by inhibiting house fly (*Musca domestica* L.) detoxicative monooxygenase activity[3] and effectively synergized resmethrin in resistant strains of *M. domestica*[4] and *Spodoptera littoralis* (Boisd.).[5]

In a preliminary report, we observed that 1,2,4-trichloro-3-(2-propynyloxy)benzene was synergistic with the photostable pyrethroid permethrin in a highly resistant strain of the major cotton pest, *Heliothis virescens* (F.).[6] Although piperonyl butoxide is a more common diagnostic synergist for the monooxygenase mechanism, piperonyl butoxide was ineffective in our strains. Forrester *et al.*[7] found our sample of 1,2,4-trichloro-3-(2-propynyloxy)benzene and piperonyl butoxide to be synergists of fenvalerate in pyrethroid-resistant *Helicoverpa armigera* (Hübner), a key Australian cotton pest.

Herein, we report full details of synergism of permethrin by 1,2,4-trichloro-3-(2-propynyloxy)benzene and related propynyl aryl ethers in permethrin-resistant

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H. virescens. Included are the cross-resistance spectra of permethrin-resistant *H. virescens* larvae, the synthesis and synergist activities of seven propynyl aryl ethers, and the diagnosis of several possible mechanisms of permethrin resistance using a diversity of synergists. In addition, the effects of 1,2,4-trichloro-3-(2-propynyloxy) benzene on the susceptibilities of these tobacco budworm larvae to DDT and methyl parathion were examined. Genetic inheritance of permethrin resistance in these strains has been described previously.[8]

2 EXPERIMENTAL METHODS

2.1 Insecticides and synergists

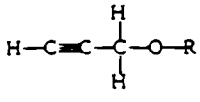
The following chemicals were provided by the manufacturers: permethrin (97.7%), *trans*-permethrin, *cis*-permethrin, cypermethrin (96.2%), fenpropathrin, fenvalerate (95%), flucythrinate (80.6%), chlorpyrifos (97.7%), methyl parathion (99%), profenofos (83.2%), sulprofos (87%), amitraz (99%), chlordimeform (93.3%), DDT (100%), endosulfan, ENT 8184 (MGK 264; *N*-(2-ethylhexyl)-8,9,10-trinorborn-5-ene-2,3-dicarboximide), piperonyl butoxide (80%), SKF-525A, *S,S,S*-tributyl phosphorotrithioate (TBPT) (95.4%), triphenyl phosphate (TPP) (98%), and *N*-2-nitrophenylcarbamic acid propynyl ester (CGA-84708) (99%). *O*-4-nitrophenyl diphenylphosphinate (DPP) was provided by the US Army Medical Research Institute of Chemical Defense, Aberdeen, Maryland, USA. Trichlorophenols (2,3,4-; 2,3,5-; 2,3,6- and 2,4,5-isomers) were purchased from Riedel de-Haën, Hannover, Germany. 2,4,6-Trichlorophenol and 3-bromopropyne were obtained from Aldrich, Milwaukee, Wisconsin, USA.

Note: older preparations of trichlorophenols might contain TCDD, a suspected carcinogen, as a by-product of synthesis.

2.2 Synthesis of propynyl aryl ethers

Six propynyl aryl ethers, synergists **1–6** (Table 1), were synthesized by the reaction of 3-bromopropyne with various alcohols.[9] Crude products from the chlorinated

TABLE 1
Synthesis of 2-Propynyloxy-Substituted Synergists



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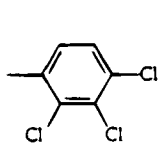

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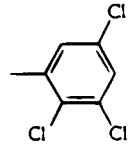

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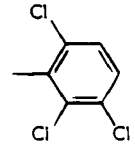

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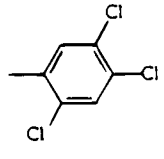

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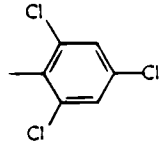

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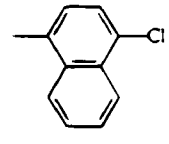

6

Synergist	*Yield* (%)	*MP* (°C)	UV_{max}(*E*, M^{-1} cm^{-1})[a]	R_t[b], *min*
1	26·9	76	206·5, 284·1 (37630, 1979)	12·8
2	79·9	60	207·5, 279·9 (29030, 1694)	15·0
3	58·8	58	206·1, 275.0 (29910, 515)	11·4
4	49·0	63	207·7, 287·1 (37160, 2092)	14·2
5	67·3	98	207·1, 278·9 (34210, 716)	15·0
6	71·6	76	212·5, 297·1 (57863, 7235)	17·6

[a] Molar extinction coefficient.
[b] HPLC retention time; Whatman C18 ODS-3; 210 nm; 1·0 ml min^{-1}; 70% methanol.

alcohols were filtered, desiccated, recrystallized twice from hot ethanol, dried overnight *in vacuo* at room temperature, and weighed to determine percentage yield. Analytical information to verify identity was obtained by mass spectrometry (MS), ultraviolet (UV), nuclear magnetic resonance (NMR), and infrared (IR) spectroscopy, and thin layer (TLC) and high performance liquid chromatography (HPLC). HPLC was performed on an octadecylsilyl-bonded, reversed phase column (4.6 × 250 mm, Whatman, Clifton, NJ) with mobile phase (methanol + water, 7 + 3 by volume) pumped at a constant flow (1 ml min^{-1}). Samples were injected in acetonitrile (2.5 μl) and detection was by UV absorption (210 nm).

2.3 *Heliothis virescens* strains

Woodrow83 was a pyrethroid-susceptible, methyl parathion-resistant strain possessing a methyl paraoxon-insensitive acetylcholinesterase, while Florence87 was fully susceptible.[10] Two pyrethroid-resistant strains (HSB-R and OC-R) were derived genetically from ICI-82.[8] The Pyrethroid-R strain was derived more recently from HSB-R and possessed the *hscp-R* gene for pyrethroid resistance in a Woodrow83 background.[11]

2.4 Susceptibility testing

Solutions of insecticides and synergists in glass-distilled acetone were applied topically to fourth-instar, 35-mg larvae as described previously.[8] Ten larvae were tested per dose for each treatment in one replicate, and four replications were performed. Mortalities were assessed at 48 h post-treatment. Larvae were considered dead if they failed to move across the diet when probed. Median lethal dose (LD_{50}) values were computed by probit analysis using SAS® (1979, SAS Institute, Raleigh, North Carolina, USA). LD_{50} values were considered significantly different by non-overlap of the LD_{50} fiducial limits.

To determine synergistic activity, percentage mortality values for each insecticide and insecticide + synergist treatment were transformed to probability units, and probit values determined for insecticide mortality were subtracted from probit values determined for insecticide: synergist mortality. The hypothesis that the toxicity of permethrin (71.43 $\mu g\ g^{-1}$) and the toxicity of permethrin + synergist (1 : 20 or 1 : 1) treatments are not significantly different was tested by calculating chi square values from the observed and expected mortality values. There was no mortality in acetone-treated control insects in any test.

3 RESULTS

3.1 Synthesis of propynyl aryl ethers

Melting points for 2-propynyloxy-substituted synergists **1**–**6** (Table 1) were similar to those reported for the original syntheses;[2] however, yields (herein calculated for the recrystallized product) ranged from 26.9% to 79.9% which were lower than reported elsewhere.[9]

Synthesis of 1,2,4-trichloro-3-(2-propynyloxy)benzene (**3**) was confirmed by the following spectral analyses which to our knowledge have not been published previously: (A) MS demonstated a molecular ion peak at $m/z = 234$ consistent with the molecular weight and characteristic isotope peaks at $m/z = 236$ and $m/z = 238$ (Fig. 1A). Percentage abundances of the M, M + 1, and M + 2 isotopes supported the proposed trichlorinated structure. The base peak ($m/z = 199$) resulted from the loss of ^{35}Cl—. The peak cluster at $m/z = 167$ to 171 suggested the loss of $—CH_2C{\equiv}H$ and CO rearrangement of the proposed trichlorinated phenyl moiety to a trichlorinated cyclopentadienyl cation. (B) Proton NMR spectroscopy resonances corresponding to the aromatic (7.24, br.s., 2H), methylene (4.80, d., J = 2.7 Hz, 2H), and terminal (2.55, t., J = 2.7 Hz, 1H) protons were shown (Fig. 1B). (C) IR spectroscopic identification confirmed the previous spectra (Fig. 1C). Absorption bands at approximately 3300 cm^{-1} and 3000 cm^{-1} corresponded to C—H stretching at the terminal acetylene and aromatic hydrogens. Broad absorption bands at 1750 cm^{-1} to 1700 cm^{-1} were characteristic of a 1,2,3,4-tetrasubstituted benzene. Results were consistent with those obtained by NMR. Other characteristic peaks at 1450 cm^{-1}, 1420 cm^{-1} and 1370 cm^{-1} represented double bond stretching, methylene scissoring, and asymmetric ether stretching, respectively.

Having confirmed by spectral analyses that the product of the initial synthesis was **3**, it was assumed that syntheses of the remaining compounds gave the theoretical products; they were analyzed only for melting point, UV absorbance, and purity by TLC and HPLC. All synthesized compounds displayed strong UV absorbance, differed in TLC retention from the reactants, and produced single peaks by HPLC analysis.

3.2 Resistance of *Heliothis virescens* strains

HSB-R and OC-R larvae were highly resistant to permethrin, the pyrethroid insecticide used to select for resistance in the laboratory (Table 2). Cross-resistance to other pyrethroids was in the order: fenpropathrin > fenvalerate > flucythrinate > cypermethrin. With the exception of fenpropathrin, these α-cyano-

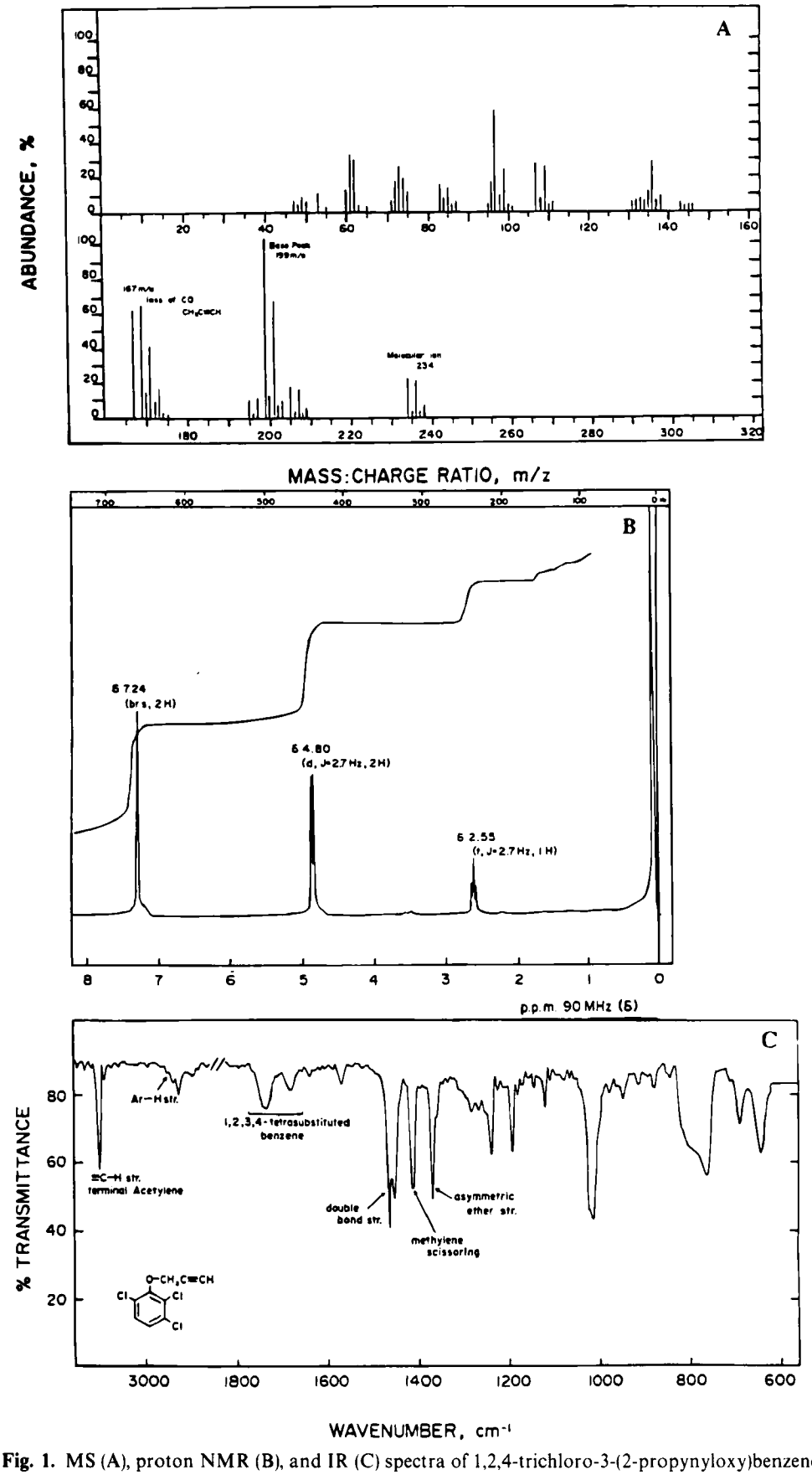

Fig. 1. MS (A), proton NMR (B), and IR (C) spectra of 1,2,4-trichloro-3-(2-propynyloxy)benzene.

TABLE 2
Resistance to Pyrethroid Insecticides in *Heliothis virescens* Larvae

Strain	*Treatment*	LD_{50} ($\mu g\ g^{-1}$)	*Slope*	*RR*[a]
Woodrow83	Permethrin	0·91	1·80	—
	Cypermethrin	1·57	2·42	—
	Fenpropathrin	5·54	2·84	—
	Fenvalerate	1·88	2·49	—
	Flucythrinate	2·09	2·49	—
HSB-R[b]	Permethrin	490·71	1·54	539·24
	Cypermethrin	46·56	2·80	29·66
	Fenpropathrin	2751·45	1·38	496·65
	Fenvalerate	353·54	0·86	188·05
	Flucythrinate	261·87	1·18	125·30
OC-R[b]	Permethrin	498·38	1·29	541·08
	Cypermethrin	14·61	0·71	9·30
	Fenpropathrin	>2857[c]	—	—
	Fenvalerate	524·27	0·63	278·87
	Flucythrinate	80·91	0·83	38·71

[a] Resistance ratio: LD_{50} divided by the LD_{50} of Woodrow83 strain.
[b] Strain selected for resistance by exposure to permethrin.
[c] The highest dose of fenpropathrin applied (2857 $\mu g\ g^{-1}$) killed 15% of the larvae treated.

pyrethroids were typically more toxic than permethrin to the HSB-R and OC-R larvae; however, they were less potent than permethrin to Woodrow83 larvae (Table 2). Resistance to fenvalerate in HSB-R and OC-R strains was characterized by a very heterogeneous response as indicated by shallow slopes of the dosage–mortality lines. Woodrow83 larvae exhibited a more homogeneous response to the α-cyano-pyrethroids.

Although susceptible to pyrethroids, the Woodrow83 strain was highly resistant to methyl parathion (Table 3). The HSB-R and OC-R strains were less resistant to methyl parathion, but 2- to 5-fold more tolerant than Woodrow83 to three other organophosphorus (OP) insecticides. The *cis*-isomer of permethrin was 4.2-fold and 3.3-fold more toxic than *trans*-permethrin against the HSB-R and OC-R strains (Table 4). OC-R larvae were 1.9-fold and 1.6-fold more tolerant to the *cis*- and *trans*-isomers, as compared to HSB-R larvae.

3.3 Activity of synergists

Prop-2-ynyl aryl ethers (synergists **1**–**6**) were the only potent synergists of permethrin among 16 candidates evaluated (Table 4). The most effective was **3**, and the least effective was **6**. Other monooxygenase inhibitors

TABLE 3
Resistance to Organophosphorus Insecticides in *Heliothis virescens* Larvae

Strain	*Treatment*	LD_{50} ($\mu g\ g^{-1}$)	*Slope*	*RR*[a]	*RR*[b]
Woodrow83	Chlorpyrifos	107·11	2.21	—	
	Methyl parathion	2623·89	0·80	—	835·63
	Profenofos	8·28	1.29	—	4·63
	Sulprofos	28·72	1·55	—	2·18
HSB-R[c]	Chlorpyrifos	162·82	3·03	1·52	
	Methyl parathion	656·61	1·13	0·25	209·11
	Profenofos	31·70	1·60	3·83	17·71
	Sulprofos	94·62	2·30	3·30	7·17
OC-R[c]	Chlorpyrifos	231·43	1·78	2·16	
	Methyl parathion	628·57	0·99	0·24	200·18
	Profenofos	41·68	2·08	5·03	23·28
	Sulprofos	87·06	1·71	3·03	6·60

[a] Resistance ratio: LD_{50} divided by LD_{50} of the Woodrow83 strain.
[b] Resistance ratio: LD_{50} divided by the LD_{50} of Florence 70 strain.[32]
[c] Strain selected for resistance by exposure to permethrin.

TABLE 4
Activity of Insecticides and Synergists in *Heliothis virescens*

Treatment	Mortality (%)			
	Woodrow83[a]	*HSB-R*[b]	*OC-R*[b]	*Permethrin R*[c]
Permethrin[d]	—	26	35	12
Permethrin[d] plus monooxygenase inhibitors:				
+ Piperonyl butoxide (1:20)	—	5[e]	8[e]	—
+ Piperonyl butoxide (1:1)	—	25	22	—
+ ENT 8184 (1:20)	—	0[e]	2[e]	—
+ SKF-525A (1:20)	—	2[e]	8[e]	—
+ Synergist **1** (1:20)	—	58[e]	70[e]	—
+ Synergist **2** (1:20)	—	60[e]	75[e]	—
+ Synergist **3** (1:20)	—	85[e]	97[e]	45
+ Synergist **4** (1:20)	—	57[e]	78[e]	—
+ Synergist **5** (1:20)	—	78[e]	80[e]	—
+ Synergist **6** (1:20)	—	52[e]	44	—
Permethrin[d] plus formamidine insecticides:				
+ Amitraz (1:20)	—	—	0[e]	—
+ Chlordimeform (1:20)	—	31	48	—
+ Chlordimeform (1:1)	—	20	30	—
Permethrin[d] plus carboxylester hydrolase inhibitors:				
+ TBPT (1:20)	—	0[e]	10[e]	—
+ TPP (1:20)	—	10[e]	0[e]	—
+ DPP (1:20)	—	—	0[e]	—
cis-Permethrin[f]	—	97	50	—
trans-Permethrin[f]	—	23	15	—
Endosulfan[g]	20	30	30	—
DDT[g]	20	—	0	—
DDT[g] plus:				
+ Synergist **3** (1:1)	74	—	24	0
+ Chlorfenethol (1:1)	40	—	0	0
Methyl parathion[h]	29	—	23	0
Methyl parathion[h] plus:				
+ Synergist **3** (1:5)	75	—	25	—
+ TBPT (1:5)	91	—	35	—

[a] Permethrin-susceptible.
[b] Permethrin-resistant *H. virescens*.
[c] Permethrin-resistant *H. virescens* derived from HSB-R with Woodrow83 background.
[d] 71·4 $\mu g\ g^{-1}$.
[e] $\chi^2 > 3{\cdot}841$, df = 1, $P = 0{\cdot}05$; significantly greater or less than mortality from permethrin alone.
[f] 231·4 $\mu g\ g^{-1}$.
[g] 2857 $\mu g\ g^{-1}$.
[h] 400 $\mu g\ g^{-1}$.

such as piperonyl butoxide, ENT 8184, and SKF-525A did not synergize the action of permethrin (Table 4). Instead, these (1:20) mixtures were antagonistic.

Confirmation that **3** was a potent synergist of permethrin was observed with serial dilutions and dosage–mortality regression analysis (Table 5). Synergism was 34- and 25-fold in pyrethroid-resistant HSB-R and OC-R strains, but only 1.4-fold in pyrethroid-susceptible larvae. Synergist activity declined as the ratio of **3** to permethrin was decreased in the mixture. The propynyl ester, CGA-84708, was a moderate synergist of permethrin when administered in 20-fold excess to resistant larvae. CGA-84708 was antagonistic to permethrin in susceptible larvae (Table 5).

Neither the formamidine candidates amitraz and chlordimeform, nor the organophosphorus candidates DPP, TPP, and TBPT, were synergists for permethrin in this study (Table 4). Although permethrin + chlorpyrifos (1:1) was 4.7-fold more toxic than permethrin in OC-R larvae (Table 5), chlorpyrifos was not an effective synergist of permethrin because this mixture was no more toxic than chlorpyrifos alone (Table 3). Chlorpyrifos was an antagonist of permethrin when this mixture was tested in Woodrow83 larvae (Table 5).

TABLE 5
Synergism of Permethrin in *Heliothis virescens* larvae

Strain	*Treatment*[a]	LD_{50} ($\mu g\ g^{-1}$)[b]	*Slope*	*SR*[c]
Woodrow83[d]	Synergist **3** (1:20)	0·65	2·04	1·40
	CGA-84708 (1:20)	4.51	2.82	0.20
	Chlorpyrifos (1:1)	1·61	1·90	0.56
HSB-R[e]	Synergist **3** (1:20)	14·58	1·82	33·66
OC-R[e]	Synergist **3** (1:20)	19·40	1·69	25·38
	Synergist **3** (1:10)	83·17	1·13	5·92
	Synergist **3** (1:1)	nm[f]		
	CGA-84708 (1:20)	69·06	1·48	7·13
	Chlorpyrifos (1:1)	104·71	1·73	4·70

[a] Mixture of permethrin plus candidate synergist; synergist **3** and CGA-84708 were not toxic alone at the doses administered.
[b] Values represent μg permethrin component of mixture per g larva.
[c] Synergistic ratio: LD_{50} of permethrin alone (*vide* Table 2) divided by the LD_{50} of permethrin: synergist mixture.
[d] Permethrin-susceptible *H. virescens strain.*
[e] Permethrin-resistant *H. virescens* strains.
[f] No mortality observed at the highest dose administered (71·43 $\mu g\ g^{-1}$).

In addition to being a synergist of permethrin in the HSB-R and OC-R strains, **3** increased the activity of DDT in both the Woodrow83 strain and the OC-R strain (Table 4); **3** was more effective than chlorfenethol which synergized DDT in the Woodrow83 strain, but not in the OC-R strain (Table 4); and although less effective than the hydrolase inhibitor TBPT, **3** was a synergist of methyl parathion in the Woodrow83 strain and the OC-R strain (Table 4).

When applied alone, all proposed synergistic compounds examined, except chlorpyrifos, were non-toxic at the doses administered. The OC-R strain was more susceptible than the HSB-R strain to permethrin + synergist treatments.

4 DISCUSSION

Prop-2-ynyl aryl ethers were the most active of any synergist chemistry examined in partially negating permethrin resistance in the HSB-R and OC-R strains. These compounds are reported to be potent monooxygenase (MO) inhibitors[3,12,13] Little *et al.*[14] found a high metabolic turnover of *trans*-cypermethrin via a monooxygenase mechanism in permethrin-resistant *H. virescens.* It is likely that monooxygenase inhibition was the mechanism of synergism by prop-2-ynyl aryl ethers in these insect strains, and that MO-resistance was working in concert with reduced sensitivity of the pyrethroid target, the sodium ion channel of the nerve.

The prop-2-ynyl aryl ethers were active synergists of permethrin, while the more conventional monooxygenase inhibitors piperonyl butoxide, SKF-525A and ENT 8184 were unexpectedly inactive. Among the prop-2-ynyl aryl ethers tested, the most active compounds were **3** and **5**, both of which possessed di-*ortho* aryl halogenation. Synergism of carbaryl by 25 substituted phenyl prop-2-ynyl ethers in house flies indicated that **3** was the most effective of the trichlorophenyl series, and among the three most active tested.[9]

Synergist **3** was highly effective with permethrin in both pyrethroid-resistant strains, but practically inactive in the pyrethroid-susceptible Woodrow83 strain (Table 5). These data suggest that this synergist is acting upon a monooxygenase activity associated with pyrethroid resistance that is insensitive to the effects of piperonyl butoxide and is in contrast to previous reports in which piperonyl butoxide was synergistic with cypermethrin[15] and fenvalerate[16] in both resistant and susceptible insects.

The monooxygenase in these pyrethroid-resistant *H. virescens* may be unusual in its specific sensitivity to prop-2-ynyl aryl ethers (Table 4). Synergism of fenvalerate by our sample of **3** was observed in pyrethroid-resistant *H. armigera*; however, piperonyl butoxide was also effective in this species.[7] Piperonyl butoxide and the *N*-alkyl synergists, ENT 8184 and SKF-525A, have been effective synergists of pyrethroid insecticides in resistant Lepidoptera;[17–20] however, none was an effective synergist of permethrin in HSB-R and OCR larvae (Table 4). Multiple forms of P450 exist in insects,[21] but relative sensitivities of these forms to inhibitors are unknown. A similar specificity may exist for a monooxygenase of resistant *Plutella xylostella* (L.), in which 4-chloro-1-naphthyl propynyl ether (**6**) was a more effective synergist of the thiazole SN72129 then either ENT 8184 or piperonyl butoxide.[22]

Two resistance mechanisms, both monooxygenase

detoxication and reduced sodium ion channel sensitivity, are likely to be present in these resistant *H. virescens* strains. Genetic crosses revealed an incompletely recessive factor indicative of a *kdr*-type mechanism in the HSB-R and OC-R strains,[8] and neurophysiological evidence for nerve insensitivity in these strains has been demonstrated.[23] High levels of DDT resistance, suppressable by neither **3** nor chlorfenethol (Table 4), and broad pyrethroid cross-resistance (Table 2) supported the presence of this non-detoxicative mechanism. Many previous cases of resistance have been attributed to this *kdr*-type mechanism,[24] which might be expected in *H. virescens* from past exposure and resistance to DDT followed by intense selection with permethrin.

Lack of permethrin synergism by organophosphorus compounds such as TBPT (Table 4) and chlorpyrifos (Table 5) suggested that permethrin hydrolysis was of little importance in HSB-R and OC-R larvae. Previously, this mechanism conferred low-level resistance to permethrin in *H. virescens* from Louisiana.[25] Although profenofos has been reported as a pyrethroid synergist,[26] it was ineffective in *H. armigera*,[7] and it was not tested by us. On the other hand, TBPT synergism of methyl parathion in Woodrow83 (Table 4), Florence79,[27] and NC-86[28] *H. virescens* strains indicated that detoxicative hydrolysis was an important organophosphorus resistance mechanism. Clearly, this hydrolytic mechanism did not confer cross-resistance to pyrethroids in the HSB-R and OC-R strains.

Conversely, the oxidative mechanism in pyrethroid resistance did not provide full methyl parathion resistance as seen in the organophosphorus-resistance spectrum of HSB-R and OC-R strains (Table 3). There was only one-fourth the level of methyl parathion resistance, but greater resistance of chlorpyrifos, profenofos, and sulprofos. The effect of monooxygenase inhibitors on organothiophosphates is complicated by the fact that, in addition to detoxication, bioactivation to the oxon metabolite is mediated by monooxygenases.

Although formamidine mixtures with pyrethroids as films inside glass vials have been shown to be synergistic against *H. virescens* larvae,[29] chlordimeform did not synergize permethrin toxicity in HSB-R and OC-R larvae by topical application (Table 4). Likewise, formamidines were not effective synergists in *H. armigera*.[7] The proposed modes of synergistic action of formamidines are inhibition of monooxygenase activity[4,30] and increased specific binding of DDT and pyrethroids to target tissue receptors.[31]

Conclusions from this study of high levels of resistance to permethrin in *H. virescens* larvae were: (a) only one class of monooxygenase inhibitor, the propynyl aryl ethers, were potent synergists of permethrin in pyrethroid-resistant strains, indicating oxidative detoxication via an unusual monooxygenase as a pyrethroid resistance mechanism; (b) non-detoxicative DDT cross-resistance and broad pyrethroid cross-resistance supported previous evidence for target insensitivity as a second mechanism; and (c) hydrolytic detoxication, important in methyl parathion-resistant strains, appeared to have little or no role in pyrethroid-resistant strains. Further biochemical investigations are necessary in order to confirm the activity of these prop-2-ynyl aryl ether synergists as monooxygenase inhibitors in these permethrin-resistant strains of *H. virescens* larvae.

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